Antimicrobial Activity of Sulfur Compounds Derived from Cabbage‡

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ABSTRACT

Selected sulfur compounds found in cabbage and its fermentation product, sauerkraut, were tested for minimum inhibitory concentration (MIC) against growth of 15 species of bacteria and 4 species of yeasts. *S*-Methyl-L-cysteine sulfoxide, sinigrin, and dimethyl sulfide at 500 ppm were not inhibitory to any of the bacteria and yeasts tested. Dimethyl disulfide at 500 ppm retarded some, but did not prevent growth of any of the test microorganisms. Dimethyl trisulfide had an MIC to bacteria of 200 ppm and to yeast of 20 ppm. Methyl methanethiosulfinate had an MIC between 50 and 200 ppm for all bacteria, and between 6 and 10 ppm for all yeasts tested. Methyl methanethiosulfonate had an MIC between 20 and 100 ppm for bacteria and between 50 and 500 ppm for yeasts. Allyl isothiocyanate had an MIC between 50 and 500 ppm for bacteria and between 1 and 4 ppm for yeasts. Methyl methanethiosulfinate was 10 to 100 times more inhibitory against *Listeria monocytogenes* at pH values of 5, 6, and 7 and was much less influenced by pH than was sodium benzoate.

Key words: Sulfur, cabbage, antimicrobial, methyl methanethiosulfinate, Listeria monocytogenes

Various sulfur compounds have been found in cabbage and/or sauerkraut, including sinigrin, allyl isothiocyanate (AITC), *S*-methyl-L-cysteine sulfoxide (SMCSO), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), methyl methanethiosulfinate (MMTSO), dimethyl sulfide (DMS) and methyl methanethiosulfonate ($MMTSO_2$) *(5, 8, 9, 12, 13, 17, 18, 22, 26)*. These investigations were made mostly from the standpoint of flavor.

MMTSO, the primary breakdown product of SMCSO *(26)*, has been shown to be degraded into volatile sulfur compounds, including $MMTSO_2$, DMDS, and DMTS *(9, 26)*. MMTSO belongs to the same chemical group, thiosulfinates, as allicin, an antimicrobial compound in garlic *(6, 7, 29, 30)*. The principal antibacterial activity of fresh cabbage juice was shown to be due to MMTSO generated from SMCSO, presumably by the action of SMCSO lyase *(16)*. MMTSO appeared in unheated cabbage juice after incubation at 30°C for 6 h and reached a maximum concentration at about 24 h, after which the concentration declined. $MMTSO_2$ is another strongly antimicrobial compound *(30)* which is generated by a spontaneous disproportionation reaction of MMTSO *(9)*. We found that $MMTSO_2$ is formed when cabbage juice is heated *(18)*. SMCSO has been reported in cabbage at concentrations of 185 to 2,218 ppm *(2, 16, 26, 28, 35)*. Mae et al. *(24)* reported that Chinese cabbage contained up to 786 ppm of SMCSO on a fresh weight basis and presumed that SMCSO played an important role in sulfur metabolism by acting as a soluble pool for organic sulfur.

Isothiocyanates, generated from glucosinolates by the action of thioglucosidase (myrosinase), have been reported to be antimicrobial *(38)*. Among the isothiocyanates, AITC has been reported to be in variable amounts in cabbage and to be responsible for the pungent flavor of fresh cabbage *(8, 15, 17, 21)*. Extensive research has been done on various types of glucosinolates in *Brassica* spp. vegetables *(14, 37)*.

The objectives of this investigation were to determine the MIC of selected sulfur compounds found in cabbage against bacteria and yeasts, and to test the effect of pH on the relative inhibitory activity of MMTSO and sodium benzoate against *Listeria monocytogenes*. The possibility of using some of the natural sulfur compounds of cabbage and other *Brassica* spp. as new food preservatives was considered.

MATERIALS AND METHODS

Materials

Sinigrin, $MMTSO_2$, *S*-methyl-L-cysteine, and DMDS were purchased from Sigma Chemical Company (St. Louis, MO). AITC, peracetic acid, and DMS were obtained from Aldrich Chemical Company (Milwaukee, WI), and sodium benzoate was from Fisher Scientific (Pittsburg, PA). DMTS (Eastman Kodak Company, Rochester, NY) was provided by Dr. R. C. Lindsay, Department of Food Science, University of Wisconsin (Madison, WI).

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SMCSO preparation

SMCSO was synthesized by the method of Lepp and Dunn *(19)* by oxidizing *S*-methyl-L-cysteine. The (+) diastereoisomer was separated from the (−) form by fractional crystallization from an acetone-water mixture or water-ethanol mixture *(34, 39)*. SMCSO was confirmed by mass spectrometry using fast atom bombardment *(16)*.

MMTSO preparation

MMTSO was synthesized by oxidizing DMDS with peracetic acid according to the method of Moore and O'Connor *(27)*. It was purified by vacuum distillation at 2 mm Hg (ca. 266 Pa), and the fraction boiling at 65°C was collected *(9)*. It was 96% pure as analyzed by gas chromatography-mass spectrometry. Prepared MMTSO was stored at −83°C between use.

MIC determination for sulfur compounds

The antimicrobial activity of test compounds for bacteria and yeasts (see Table 1) was tested in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) with 2% added glucose and yeast morphology broth (YMB) (Difco), respectively, using the MIC procedure described by Brock and Madigan *(3)*. Each test compound was dissolved in heat-sterilized appropriate medium, filter-sterilized, and 10-ml aliquots were dispensed into 16 by 150 mm culture tubes with caps. Initial concentrations of the test compounds were varied between 100 and 1,000 ppm in increments of 100 ppm. When a test microorganism did not grow at 100 ppm, concentrations were varied between 10 and 100 ppm in 10-ppm increments. If no growth occurred at 10 ppm, concentrations were varied between 1 and 10 ppm in 1-ppm increments. The media were inoculated with bacteria and yeasts to make an initial cell population of approximately 10^5 cells per ml and statically incubated. Complete absence of visual turbidity after incubation for 48 h at 30°C was regarded as an indication of no growth. We found 48 h to be sufficient time for growth of bacteria and yeasts to produce visible turbidity in the absence of added test compounds to either TSB (bacteria) or YMB (yeasts). The TSB (pH 7.3, unadjusted) was adjusted to the desired pH with HCl for comparing the relative efficacy of MMTSO and sodium benzoate for inhibition of *L. monocytogenes*.

Microbial test cultures

Bacteria and yeast cultures were maintained in the U.S. Food Fermentation Laboratory culture collection. Cultures were stored at −84°C in basal media containing 16% glycerol. The basal media were MRS broth (Difco) for lactic acid bacteria, TSB for other bacteria, and YMB for yeasts. For resuscitation, the cultures were streaked onto agar medium of the same composition as used for growing and an isolated colony picked and cultivated at least two times in the growth medium before using a 16-h culture for final inoculation. The *L. monocytogenes* F5069 culture was serotype 4b, and was obtained from C. Donnelly (University of Vermont).

RESULTS AND DISCUSSION

SMCSO and its degradation products

SMCSO, MMTSO, and other sulfur compounds (DMS, DMDS, DMTS, $MMTSO_2$) which are known to be generated from MMTSO were tested for their antimicrobial activity (Table 1). SMCSO, the nonvolatile precursor compound of MMTSO, was not inhibitory (did not prevent growth as determined by visual turbidity) against test microorganisms at up to 1,000 ppm, implying that SMCSO

TABLE 1. *MIC (ppm) of different sulfur compounds against selected bacteria and yeasts*

Microbial strain	MIC (ppm) of:[a]							
	SMCSO	DMS	DMDS	DMTS	MMTSO	Sinigrin	AITC	$MMTSO_2$
Bacteria[b]								
Pediococcus pentosaceus LA3 (ATCC 43200) FFL48	>1000	>500	>500	>500	200	>1000	300	100
Pediococcus pentosaceus LA76 (ATCC 33316)	>1000	>500	>500	>500	200	>1000	400	50
Leuconostoc mesenteroides LA10 (C33) FFL44	>1000	>500	>500	300	100	>1000	500	100
Leuconostoc mesenteroides LA113 (ATCC 10882)	>1000	>500	>500	>500	200	>1000	500	20
Lactobacillus plantarum LA97	>1000	>500	>500	200	50	>1000	300	50
Lactobacillus plantarum LA70 (ATCC 14917)	>1000	>500	>500	300	50	>1000	400	50
Lactobacillus brevis LA25 (MD 42)	>1000	>500	>500	200	100	>1000	300	50
Lactobacillus brevis LA200 (ATCC 8287)	>1000	>500	>500	>500	100	>1000	300	50
Listeria monocytogenes B67 (F 5069)	>1000	>500	>500	>500	50	>1000	200	50
Listeria monocytogenes B70 (ATCC 19115)	>1000	>500	>500	300	50	>1000	200	50
Staphylococcus aureus B31	>1000	>500	>500	>500	50	>1000	100	50
Escherichia coli B34 (ATCC 33625)	>1000	>500	>500	>500	50	>1000	50	50
Enterobacter aerogenes B146 (ATCC 13048)	>1000	>500	>500	>500	200	>1000	300	50
Bacillus subtilis B96	>1000	>500	>500	200	50	>1000	50	20
Salmonella typhimurium B38	>1000	>500	>500	>500	50	>1000	100	50
Yeasts[c]								
Saccharomyces cerevisiae Y6	>1000	>500	>500	20	10	>1000	4	50
Torulopsis etchellsii Y24 (Y 6651)	>1000	>500	>500	20	10	>1000	1	50
Hansenula mrakii Y27	>1000	>500	>500	20	6	>1000	4	500
Pichia membranefaciens Y20 (Y 1617)	>1000	>500	>500	20	8	>1000	2	500

[a] SMCSO, *S*-methyl-L-cysteine; DMS, dimethyl sulfide; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide; MMTSO, methyl methanethiosulfinate; AITC, allyl isothyocyanate; $MMTSO_2$, methyl methane thiosulfonate

[b] Bacteria tested in TSB with 2% added glucose.

[c] Yeasts tested in YM broth.

itself is not antimicrobial and that the microorganisms did not degrade it to inhibitory compounds. Fresh cabbage has been reported to contain up to 2,200 ppm of SMCSO *(2, 16, 26, 35)* and up to 3.3 ppm and 2.9 ppm of DMDS and DMTS, respectively, in the headspace of disrupted fresh cabbage *(8)*. SMCSO did not inhibit growth of *Leuconostoc mesenteroides* C33 *(16)*. Alliin, which belongs to the same chemical group, *S*-alk(en)yl cysteine sulfoxide, as SMCSO, and the precursor of allicin were reported not to be antimicrobial agents *(4, 33)*. MMTSO was the most inhibitory compound of all SMCSO degradation products tested, with an MIC between 50 and 200 ppm for various bacteria, including gram$^+$, gram$^-$, lactic acid, and pathogenic bacteria, and less than or equal to 10 ppm for yeasts. We found one extract of fresh cabbage to contain 84 ppm of MMTSO (data not shown). Extracts from some varieties of cabbage may contain relatively low concentrations of MMTSO since Conner et al. *(11)* found that *L. monocytogenes* grew well in autoclaved extracts, or perhaps autoclaving as they did reduced the level of MMTSO in the extracts. We found an MIC of MMTSO for *L. monocytogenes* of 50 ppm (Table 1). $MMTSO_2$ was the most inhibitory compound of all sulfur compounds tested for bacteria with an MIC between 20 and 100 ppm, but it was less inhibitory for yeasts, with an MIC between 50 and 500 ppm. Small et al. *(30)* reported that $MMTSO_2$ was equally or more antimicrobial than MMTSO. DMTS was moderately inhibitory against bacteria, but strongly inhibitory against yeasts. *L. mesenteroides* C33 was unable to grow in MRS broth in the presence of 200 ppm MMTSO *(16)*. MMTSO and allicin have been shown to be antimicrobial *(6, 7, 30)* due to their -S(O)-S- group, which is believed to react with the -SH group of cysteine and proteins to generate mixed disulfides (R-S(O)-S-R′ + HS-R″ → R′-S-S-R″ + RSOH) *(7, 29)*. The antimicrobial activity of thiosulfinates is inactivated by cysteine *(7, 29, 30)*. DMTS had an MIC equal to or greater than 200 ppm against bacteria and 20 ppm against yeasts. DMDS, which at 500 ppm was slightly inhibitory to the growth of certain microorganisms, did not completely inhibit the growth of any microorganisms within 48 h of incubation. However, the onset of growth was delayed. Disulfides such as diallyl disulfide and DMDS have been shown to be antimicrobial *(4, 38)*. The activity, however, was reported to be weaker than that of the corresponding thiosulfinates *(38)*. Theories have been proposed as to how DMDS affects cellular activities. Steven et al. *(32)* suggested that DMDS inactivates active papain by forming an inactive papain-methyl sulfide complex (papain-SH + DMDS → papain-S-S-CH_3 + CH_3SH). Smith *(31)* proposed that SMCSO is converted by rumen bacteria to DMDS, leading to the development of hemolytic anemia in the host ruminant animals feeding upon cruciferous vegetables. He explained that DMDS may be harmful to red blood cells by oxidizing reduced glutathione to oxidized glutathione (2GSH + CH_3-S-S-CH_3 → G-S-S-G + 2 CH_3SH) or by reacting with -SH groups of red blood cell membranes to give a mixed disulfide (membrane-SH + CH_3-S-S-CH_3 → membrane-S-S-CH_3 + CH_3SH). If this proposal by Smith *(31)* is true for red blood cells, the same mechanism is expected to hold for microbial cells. DMS was not antimicrobial up to 500 ppm.

Sinigrin and its degradation product

Sinigrin, the precursor of AITC, was not inhibitory to the growth of bacteria and yeasts up to 1,000 ppm (Table 1). Cabbage was reported to contain up to 145.8 ppm of sinigrin *(37)*. This implied that sinigrin itself was not antimicrobial and that microorganisms did not degrade it to its antimicrobial aglycones. AITC has been reported to be present in cabbage *(8, 15)* and sauerkraut *(12)*. AITC is known to be antimicrobial *(20, 38)* and was found herein to have an MIC between 50 and 500 ppm for bacteria, including gram$^+$, gram$^-$, pathogenic, and lactic acid bacteria. No difference in relative sensitivity toward AITC by gram$^+$ and gram$^-$ bacteria was evident.

AITC was very strongly inhibitory to the growth of both oxidative and fermentative yeasts, with an MIC of ≤4 ppm (Table 1). Cabbage was reported to contain up to 45 ppm of AITC in the headspace of disrupted fresh cabbage *(8)*. A mold (*Penicillium glaucum*) was reported to be much more sensitive to isothiocyanates, including AITC, than was a bacterium (*Staphylococcus aureus* 209 Innsbruck) *(38)*. Mycelial growth, zoospore formation, and germination of *Aphanomyces euteiches* were prevented by 0.04, 0.10, and 0.30 ppm of AITC, respectively *(20)*. It has been hypothesized that isothiocyanates are antimicrobial by reacting with -SH groups of proteins, which adversely affects cellular metabolism *(36, 40)*. Tang *(36)* proposed a reaction mechanism between papain and benzyl isothiocyanate (papain-SH + benzyl-NCS → papain-S-C(S)-NH-benzyl). Reactions between isothiocyanates and proteins were also shown at pH values higher than 6 *(1)*.

pH effect on the antimicrobial activity of MMTSO

The effect of pH on the antibacterial activity of MMTSO against *L. monocytogenes* was studied to assess its potential as a food preservative in comparison with the traditional food preservative, sodium benzoate. The antimicrobial activity of sodium benzoate very much depends upon pH of the medium, being most effective in the protonated form and, thus, at lower pH values *(10, 23)*. Figure 1 shows the MIC of benzoate and MMTSO against *L. monocytogenes* at different pH values. The MIC of benzoate was confirmed to be greatly influenced by pH, decreasing by 100-fold as the pH was decreased from 7 to 5. The MIC of MMTSO decreased only about fivefold as the pH of the medium was decreased from 7 to 5. It is believed that only the undissociated form of benzoic acid is antimicrobial *(10, 23)*. MMTSO does not have an ionizable structure, which perhaps explains why its activity is less affected by pH than is sodium benzoate.

AITC and MMTSO may have unique applications as preservatives in nonacidic minimally processed foods. Limitations to their use, however, include effects on product flavor and on human health. For example, MMTSO has been shown to inhibit genotoxicity in mice *(25)*, but 0.5 mmol per kg (55 ppm) of body weight produced severe acute toxicity *(26)*. However, these authors suggested that MMTSO may, in part, be responsible for the anticarcinogenic effect of *Brassica* vegetables.

The results of this investigation indicate that MMTSO and AITC, which occur naturally in cabbage and sauerkraut,

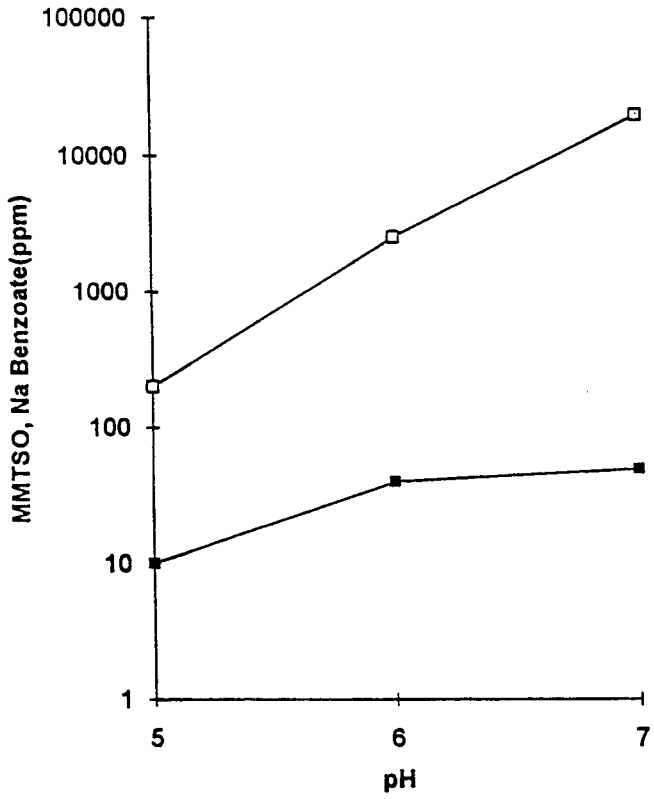

FIGURE 1. *Minimum inhibitory concentration (ppm) of MMTSO (■) and sodium benzoate (□) against L. monocytogenes F 5069 at different pH values. The basal medium for these tests was TSB.*

have very strong antimicrobial activities, being 20 to 100 times more inhibitory than sodium benzoate depending on pH (between 5 and 7). Factors influencing the generation of the two compounds may be important in regulating the fermentation of vegetables containing their precursor compounds, SMCSO and sinigrin. MMTSO and AITC may also serve as preservatives for foods of which these compounds are natural components.

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